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Focus on Applications of CRISPR

Science and Society

Genome Editing for Global Food Security

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Global food security is increasingly challenging in light of population increase, the impact of climate change on crop production, and limited land available for agricultural expansion. Here we outline how genome editing provides excellent and timely methods to optimize crop plants, and argue the urgency for societal acceptance and support.

Challenges of World Food Security in the 21st Century

A growing world population and dietary shifts associated with economic development[i] have resulted in increasing and changing demands for food. In addition to political and economic responses to these challenges, new agricultural technologies are required to minimize threats including climate change (Figure 1), and to fulfill increasing demands, for example, by improving sustainable development despite unfavorable conditions (i.e., soil degeneration, drought, flooding, and temperature extremes).

Modern agriculture has evolved into an immense and complex production chain with an ever-increasing reliance on crop optimization through soil, water, and postharvest management employing high-tech machinery and facilities. However, considering the significant investments required for implementation and maintenance of this chain, such options are not feasible for small farms in underdeveloped areas.

Breeding crops for better performance can reduce the requirement for expensive production facilities, while improving nutrition and yield. Considering the ongoing decrease in arable land area, and that the annual rate of crop yield increase has dropped by half compared with that during the green revolution as a result of emerging pathogens and rapidly changing environments, which overcome the pace of development for genetically improved crop varieties (i.e., yield barrier; compare Table 5.1 and Figure 5.1 in [1]), there is a rising demand for optimizing crop performance.

While traditional crop breeding has changed drastically during the last century, (e.g., marker-assisted selection and hybrid breeding), it remains time consuming and resource intensive. Furthermore, traditional crop breeding imprecisely selects for a few major traits (e.g., yield) while leading to the concomitant loss of other desirable ones (e.g., nutrition [2], flavor [3]). Finally, the cost of adapting elite varieties to meet local environmental conditions or to address regional (and variable) threats to major crops (Figure 1) has led to a gap between food security and availability of agrotechnologies to undertake these challenges, especially for emerging economies.

Hence, innovations in breeding technologies are absolutely necessary, although many face serious and sometimes unfair scrutiny from the public. Specifically, the incorporation of novel DNA elements into a host genome to introduce novel traits (i.e., genetically modified organisms) often faces negative public reactions[ii]. Here we focus on genome editing as a novel, elegant, and transgene-free way to improve and accelerate crop breeding.

Genome Editing-Aided Crop Optimization (GET-A-CROP) Strategies

Genome editing utilizes sequence-specific nucleases (SSNs), such as zinc finger nucleases, transcription activator-like effector nucleases, and the clustered regularly interspaced palindromic repeats (CRISPR)-associated system (Cas), which mimic mechanisms discovered in nature to introduce targeted mutations into crops (Figure 2). *In vivo,* SSNs create double-strand breaks to trigger the endogenous non-homologous end joining (NHEJ) or homology-directed repair pathways (Figure 2). CRISPR, the most recent SSN-based approach, has reduced costs tremendously while being

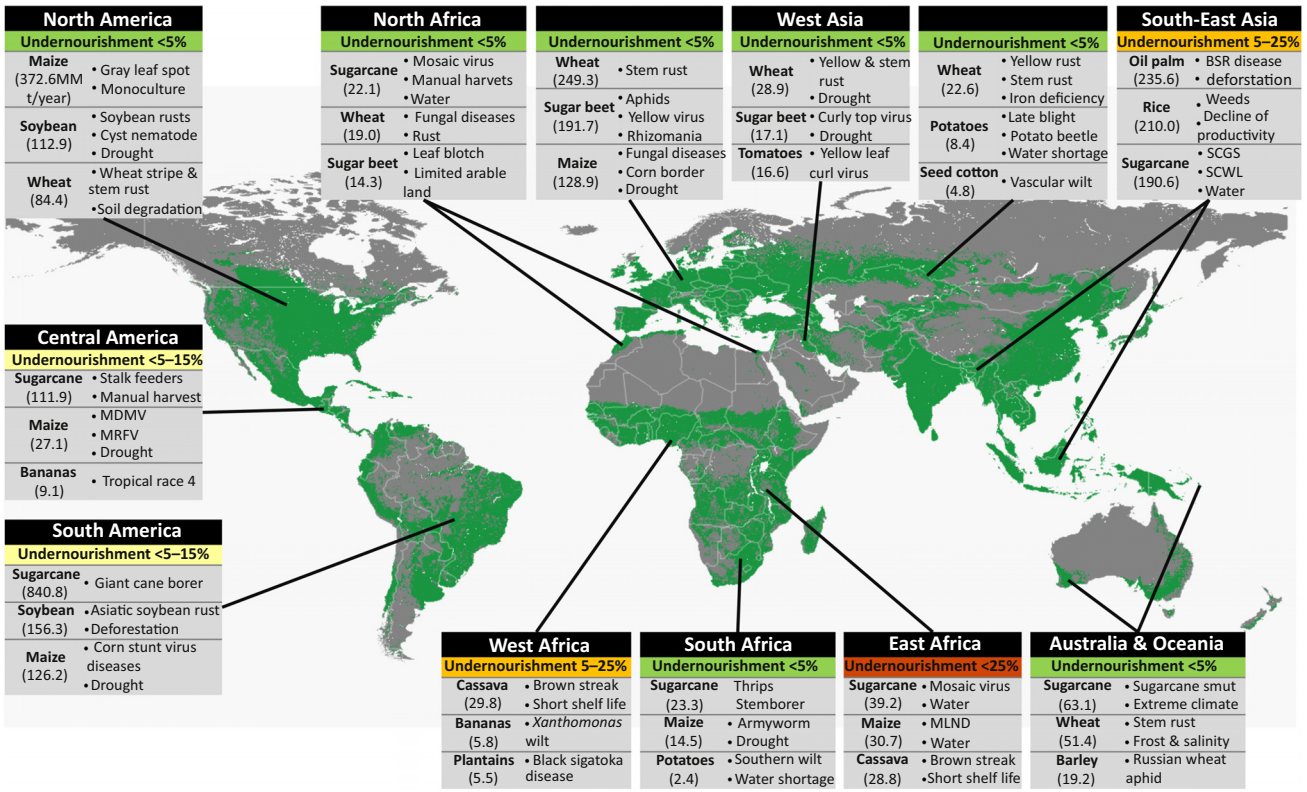

Figure 1. Global Crop Production Status and Challenges. This map displays the distribution of worldwide cropland (green color) and land not suitable or used for crop production (gray color) (adapted, with permission, from [10] and[vi]). Single charts exemplify the status of world regional undernourishment[vii], total production for major crops in millions of tons per year (MM t/year[iv]), and their prevalent biotic and abiotic threats. BSR, basal stem rot disease; MDMV, maize dwarf mosaic virus; MLND, maize lethal necrosis disease; MRFV, maize rayado fino virus; SCGS, sugarcane grassy shoot; SCWL, sugarcane white leaf.

much more efficient to become the method of choice, as is reflected in the increasing number of publications based on genome editing in plants, which also reflects the importance of these modern functional genetics tools for agricultural research [4].

A broad spectrum of targets for SSNs can be used, including gene coding sequences, regulatory elements, and epigenetic modifications. The NHEJ pathway can induce insertion–deletions (indels) leading to frameshift mutations in coding areas or fragment deletion, while the homology-directed repair pathway can introduce new DNA sequences based on foreign DNA templates. When combined with another activation, repression, or modification domain, SSNs can be applied to regulate gene expression or to modify epigenetic loci. Moreover, SSNs can be excluded from the target genome by transient expression or segregation during meiosis, thereby precluding unexpected consequences (i.e., mutations) caused by the random insertion of SSNs into the genome in subsequent generations (Figure 2) (refer to [5] for a review).

As rapid advances in phenotyping and 'omics' technologies have led to the identification of large numbers of trait-specific genes, the bottleneck in research has shifted to the validation and functional analysis of these candidate factors. In this light, technological (e.g., transformation and tissue culture) and biological (e.g., understanding gene action in different genetic backgrounds) limitations have come to the forefront of research interest. As functional genetics remains laborious, and some research efforts (e.g., the development of robust tissue culture methods) are not considered to have as much scientific impact, many promising candidate genes are neither followed up on nor validated, thus presenting a serious shortcoming to the publicly funded research behind gene discovery. Advances in genome editing present unique and cost-saving opportunities to reveal the genetic basis underlying contrasting phenotypic groups by parallel analyses of multiple candidate genes for loss-of-function and associated trait alteration. For example, genome editing opens the possibility of testing variability in gene function on a population level (i.e., in differing genetic backgrounds). Applying this method to regional crop varieties, such as cassava (Figure 2), would be especially

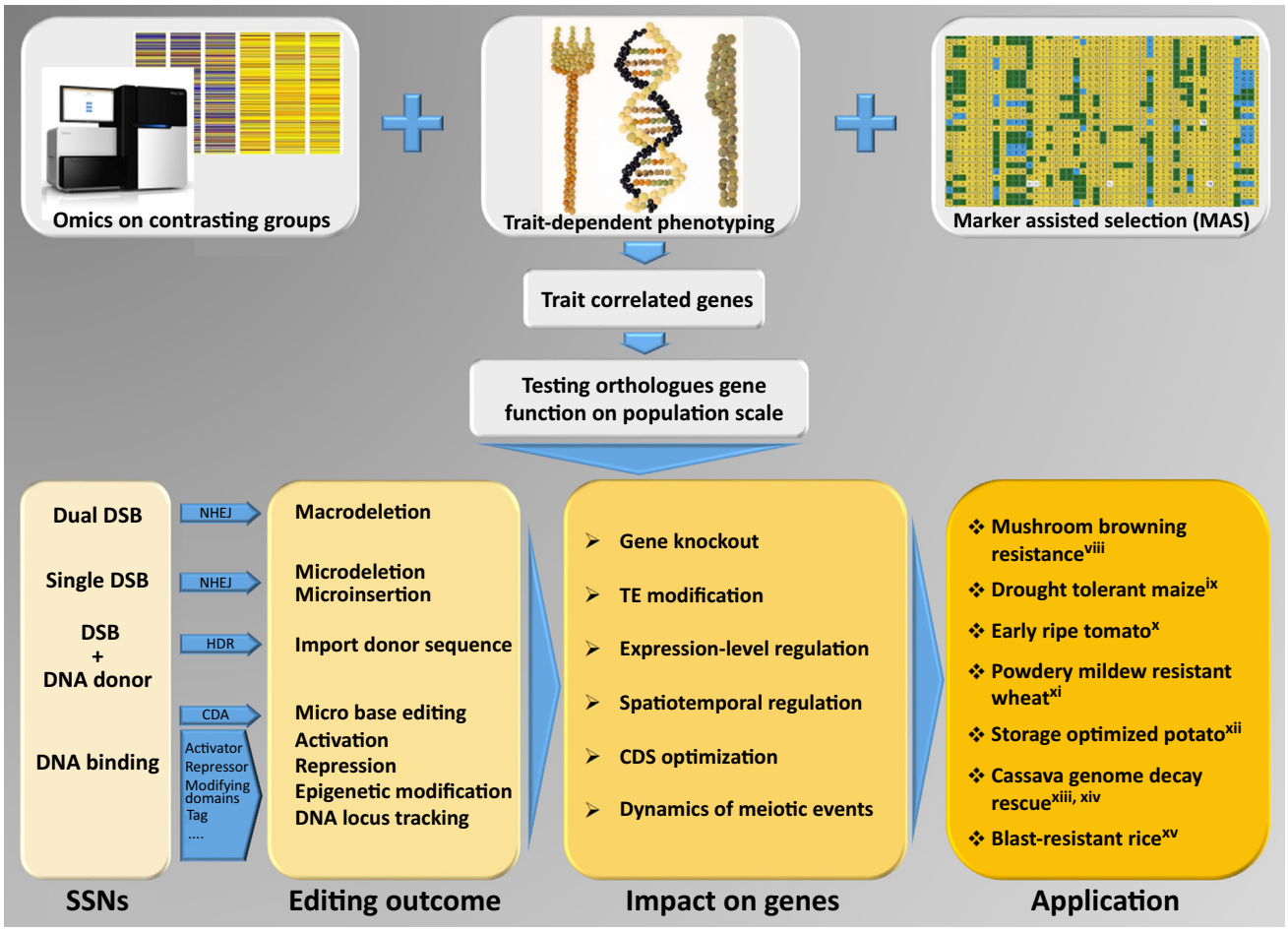

Figure 2. Genome Editing-Aided Crop Optimization (GET-A-CROP) Strategies. Once agronomically beneficial genes are identified, the currently most advanced standard procedure, called marker-assisted selection (MAS), is used to identify and implant the traits in designated crops. In the future, the more cost-effective and rapid GET-A-CROP strategies can be applied. Hereby, omics data from large contrasting phenotypic groups are used to identify trait-correlated genes. Their orthologs are then tested for functional variation on a population (i.e., testing for stability in different genetic backgrounds) scale by SSNs, which induce a broad spectrum of *in vivo* changes to multiple genomes in parallel, before applying the edited form to a designated crop plant (refer to selected examples in application box). CDA, cytidine deaminase; CDS, coding sequence; DSB, double-strand break; HDR, homology-directed repair; NHEJ, non-homologous end joining; SSNs, sequence-specific nucleases; TE, transcription element. Also see links[viii–xv] in the 'Resources' section.

advantageous considering the relative resource efficiency (i.e., money and time) and precision of trait optimization (Figures 1 and 2). In this light, this strategy could minimize the dependency on a shrinking list of major crops, which are associated with nutrition deficiency, limited genetic diversity, and lower crop resilience [6], not to mention accelerating the rate of bringing healthier orphan crops to the consumer.

Crop optimization strategies based on genome editing can aid classical breeding strategies, for example, by creating male sterile lines, and work on genes related to yield, stress tolerance, and nutrition balance [2,7], as climate change and human health (e.g., brain development in children) in developing countries are major current and future challenges. Editing multiple genes in parallel [8] furthermore facilitates the game-changing alteration of more complex traits, such as heterosis effects, or the induction of technologies that can genetically fix desired multigenic traits (e.g., apomixis, or clonal propagation via seeds).

How Genome Editing Can Serve Global Food Security

Genome editing is one of the most promising solutions for food security issues, especially in developing countries where local crop plant varieties are the mainstay. However, public acceptance of new agro-technologies in agriculture, especially in Western nations (e.g., Social License[iii]), arguably impedes their exploitation to support developing regions. Therefore, as a first step to improve the societal acceptance of genetic modification in agriculture, it is essential to cultivate

awareness of acute global food security threats – a minor issue for consumers in the Western world, but a serious topic for consumers in developing countries. The grave consequences of not implementing such technologies must be clarified to policy makers and consumers by summarizing unbiased data and overviews of historical trends (e.g., see FAO[iv]; Figure 1), as well as by offering potential solutions. Second, as social unrest with respect to resource sharing between nations comes to the forefront of global development, it is the responsibility of governments and non-governmental organizations to provide a global political framework and financial resources to assure food security and social balance within and among nations. One possibility is to mimic the concept of generic drugs for the seed market to make optimized high-tech agricultural seeds affordable to farmers (i.e., formerly patented seeds – the genetic trait and the variety – are continually provided for lower costs), but this requires both political will and industry risk[v]. Finally, we propose that agricultural research institutes make technologies and scientific discoveries visible to developing countries (Figure 2), and to provide novel, specific products (i.e., optimized agricultural seeds) through a variable licensing system that takes the affordability of farmers and users into account. In this way publicly, (or semi-privately) funded research institutes can subsidize their research, in addition to gaining trust and credit from society (social license) for addressing global food security challenges.

Concluding Remarks

Here we emphasize that global food security is an enormous challenge with multifaceted social and economic implications; it therefore requires enormous coordinated efforts within this century. While large-scale and complex agricultural production chains contribute extensively to satisfying the food supply in highly developed countries, these facilities are not equally available to less-developed nations. It is thus clear that precise crop optimization with regard to yield, nutrition balance, and plant fitness using genome editing would be a necessary strategy to address current and potential agricultural challenges (refer to Figure 1 for examples), thereby securing the food supply: investment costs for farmers can be kept low while globally diverse threats can be addressed in parallel (Figure 1). Rapid developments in genome editing technologies will decrease the costs and time required to produce optimized crops in the future, and the broad adoption of genome editing technologies for crop optimization requires government support in setting up an updated regulatory framework, which should be guided by reasonable discussion with the public [9]. In countries that follow product-based regulation (e.g., USA), only minor hurdles are expected regarding the implementation of gene-edited crops (refer to Figure 2 for examples). In regions in favor of process-based regulation (e.g., the European Union), crop varieties developed through transient expression of SSN, or merely involving NHEJ-induced several nucleotide changes and no traces of transgenic elements, should be treated similarly to varieties developed through traditional breeding. To conclude, while ethical standards and food security challenges tend to be regionally specific, the regulatory framework and legislation for gene-edited crops should follow scientific oversight, in addition to potential risk assessments and the needs of consumers and farmers on a global scale.

Acknowledgments

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Resources

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Focus on Applications of CRISPR

Science & Society

CRISPR-Based Antibacterials: Transforming Bacterial Defense into Offense

Adrienne C. Greene[1,*,@]

The development of antimicrobial-resistant (AMR) bacteria poses a serious worldwide health concern. CRISPR-based antibacterials are a novel and adaptable method for building an arsenal of antibacterials potentially capable of targeting any pathogenic bacteria.

One of the most urgent public health concerns in the USA is the rapid emergence of AMR bacteria and the arms race between AMR bacteria and the development of novel bactericidal agents. Developing antibacterials for combating AMR infections is a priority, but an obvious solution has not yet been found. According to a 2013 report by the Centers for Disease Control and Prevention, AMR bacteria infect over two million people annually and at least 23 000 of those individuals die as a result of the infection [1].

While small-molecule antibiotics are commonly used to treat bacterial infections, overuse of antibiotics has resulted in rapid bacterial adaptation and resistance to the most common US FDA-approved treatments. Furthermore, the development, screening, and testing of these antibiotics is costly and resource intensive, limiting alternative treatments against AMR bacteria. As of March 2017, there were approximately 41 new antibiotics in clinical trials in the USA [2]. The success rates of any new drug therapy, including antibiotics, are generally low; only approximately 20% of newly developed antibiotics are approved as treatments [3]. These limitations prompt the need to build an arsenal of unique antibacterials with precise targeting capabilities. To address these issues, research strategies include developing novel nucleic acid- and peptide-based antibacterials, bacteriocins, bacteriophage therapies, antibodies, and antivirulence compounds [4]. Unfortunately, these methods often require exhaustive screening to arrive at the final product of a single new antibacterial agent.

Limitations in current AMR treatments motivate a fundamental change in the approach for developing antibacterials. The overall goal of antibacterial development is to generate safe and effective treatments that can be rapidly modified and adapted to: (i) specifically target bacterial pathogens [4]; and (ii) target the same species differently to stay ahead of developing resistances. Here, we review the concept that an adaptable and potentially more-effective method for developing antibacterials is to exploit a defense system that nature has already evolved; the Type II CRISPR-Cas bacterial adaptive immune system. Recent advances in CRISPR-Cas genome engineering have made it easy to implement this technology in any laboratory. While not discussed here, there are published reviews detailing other biotechnological advances and uses of CRISPR-Cas genome engineering [5].

Within bacteria and Archaea, CRISPR-Cas exists as a mechanism to counter invasion by foreign genetic material, including mobile genetic elements and bacteriophages (or phages). CRISPR-Cas9 comprises an RNA-guided endonuclease, Cas9, which induces a double-stranded DNA (dsDNA) break. Cas9 is guided to the genomic loci of interest by a single-guide RNA containing complementary base pairs. Upon phage infection in bacteria, the Cas machinery 'barcodes' small sequences of phage genomes into the bacterial genome to counter subsequent invasion by phages [6], using CRISPR-Cas9 to cleave the foreign genetic material.

CRISPR-Cas has been extensively studied and modified to very selectively insert, delete, or mutate genes in almost any species. However, the implementation of CRISPR-based antibacterials requires advances including targeting specific pathogenic bacterial species within complex bacterial populations, delivering antibacterials to pathogenic bacteria, and, in some cases, delivering those therapeutics to host cells infected with bacterial pathogens. With the wave of research and interest in CRISPR-Cas systems currently at the scientific forefront, the novel use of CRISPR-Cas antibacterials is highlighted here.

Repurposing Bacterial Defense into Offense

One key feature of CRISPR-Cas is its highly sequence-specific targeting capability, allowing it to easily distinguish between pathogenic or commensal bacterial species. To repurpose the CRISPR-Cas machinery to attack rather than defend bacteria, CRISPR guide RNAs can be designed to target either virulence or essential chromosomal genes specific to pathogens. Induction of a dsDNA break in bacteria typically results in fatality if chromosomally targeted [7]. In short, CRISPR-Cas9 is a potential gene-editing antibacterial [8].

Delivery of CRISPR-Cas9 antibacterials poses a challenge since the active approximately 160-kDa protein–RNA complex must pass through the bacterial membrane to be effective. How then can CRISPR-Cas9 be delivered to Gram-negative and/or Gram-positive bacteria?